REMARKS

Claim 31 has been canceled. Therefore, Claims 29-30 and 32-35 remain pending. No claims are allowed.

The specification was amended at line 16 on page 13 to state that "U.S. Serial No. 09/156,954, filed September 18, 1998" is now "U.S. Patent 6,153,394 to Mansfield *et al.*"

The specification was amended at line 34 on page 29 to state that "U.S. Provisional Application Serial No. 60/120,831, filed on February 19,1999" is now "U.S. Serial No. 09/506,630, filed February 18, 2000."

Other amendments to the specification and the claims were made at the suggestion of the Examiner and are discussed below.

1. The specification was objected to for having various informalities.

The informalities have been corrected in the manner suggested by the Examiner. In particular, lines 5-10 on page 1 of the specification relating to federal sponsorship has been cancelled and the phrase "and drawings" on page 11, line 1, has been cancelled.

2. Claims 29-35 were rejected under U.S.C. § 112, first paragraph.

The applicants' believe the specification

supports the applicants' claimed method for making polyclonal and monoclonal antibodies against the 16 (±4) or 30 (±4) kDa antigens by using a fusion polypeptide which contains either the 16 (±4) or 30 (±4) kDa antigen fused to a polypeptide which enables the fusion polypeptide to be isolated by affinity chromatography.

Since the applicants have possession of *Sarcocystis neurona* which contains DNA encoding the 16 (±4) and 30 (±4) kDa antigens, the applicants have possession of the *Sarcocystis neurona* DNA encoding the 16 (±4) and 30 (±4) kDa antigens. Methods for extracting DNA from organisms, methods for making DNA expression libraries from the extracted DNA, and DNA sequencing are well known in the art. These methods have become so routine that there are automated machines that will perform each of the above methods at efficiencies and speeds that surpass the ability of any skilled artisan (for example, the machines used to sequence the human genome).

On page 27, beginning at line 23, and in Example 2, the applicants provide a simple method for isolating clones from an expression library comprising *Sarcocystis neurona* DNA which encodes the 16 (±4) or 30 (±4) kDa antigens using monoclonal antibodies against the antigen such as the monoclonal antibofies prepared as taught in Example 1. Once clones in the expression

-9-

library containing the *Sarcocystis neurona* DNA encoding the 16 (±4) or 30 (±4) kDa antigens have been identified, the genes encoding the 16 (±4) or 30 (±4) kDa antigens can be characterized merely by sequencing the DNA from the clones.

Constructing and screening an expression library for clones containing DNA encoding a particular protein is routine in the art. If one has an antibody against a particular protein or has a particular biochemical assay for detecting a particular protein, it is generally assumed that the DNA encoding the protein can be recovered with a high expectation of success. In general, the unpredictability associated with constructing and screening expression libraries to isolate genes encoding particular proteins is considered by those of ordinary skill in the art to be negligible. Therefore, because constructing and screening expression libraries is routine in the art, a person of ordinary skill in the art following the applicants' disclosure would have a high expectation of success of recovering clones from an expression library that express the 16 (±4) or 30 (±4) kDa antigens using the antibodies against the 16 (±4) or 30 (±4) kDa antigens prepared as taught in Example 1.

The applicants further teach making fusion polypeptides containing either the 16 (±4) or 30 (±4)

kDa antigen using a variety of commercially available kits. These commercial kits provide cloning vectors for making the fusion polypeptides and affinity chromatography substrates for isolating the fusion polypeptides (pages 18-20). Cloning DNA from expression libraries uses standard cloning methods which are routine and well known to those of ordinary skill in the art. In general, any unpredictability associated with constructing a vector to produce a fusion polypeptide is considered by those skilled in the art to be negligible. Therefore, a person of ordinary skill in the art would have a high expectation of success in being to construct vectors that produce fusion polypeptides using the DNA encoding the 16 (±4) or 30 (±4) kDa antigens identified as above and using the commercially available cloning vectors for making the fusion polypeptides.

Once the above vectors for producing a fusion polypeptide comprising either the 16 (±4) or 30 (±4) kDa antigen have been made, the fusion polypeptide can be isolated using the methods taught by the applicants with a high expectation of success (pages 18-20). Once the fusion polypeptide has been isolated, a person of ordinary skill in the art would have a high expectation of success of making polyclonal or monoclonal antibodies against the 16 (±4) or 30 (±4) kDa antigen comprising the fusion polypeptide.

In light of the above, because the applicants have possession of *Sarcocystis neurona*, which contains DNA encoding the 16 (±4) and 30 (±4) kDa antigens, and the methods for obtaining the nucleotide sequence of the genes encoding the 16 (±4) and 30 (±4) kDa antigens are predictable and routine in the art, the applicants constructively have possession of the sequence of the DNA encoding a fusion protein comprising the codons for the 16 (±4) or the 30 (±4) kDa antigen fused to the codons of a polypeptide which enables the fusion protein to be purified by affinity chromatography. Therefore, the specification is believed to be enabling for presently amended Claims 29, 30, and 32-35. Reconsideration of the rejection is requested.

3. Claims 29-35 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Claims 29 and 30 were rejected because the Examiner indicated that it was unclear as to what the fusion polypeptide comprises and how a polypeptide can facilitate isolation of a fusion polypeptide.

The DNA in Claims 29 and 30 is a single DNA molecule encoding a fusion polypeptide which comprises the codons for a *Sarcocystis neurona* antigen in frame with the codons for a polypeptide that enables the fusion polypeptide to be isolated by affinity

-12-

chromatography. When the DNA is transcribed and the mRNA therefrom is translated, a fusion polypeptide is produced which contains the amino acid sequence for the *Sarcocystis neurona* antigen fused to the amino acid sequence for the polypeptide.

Claims 29 and 30 have been amended to clarify that the applicants are claiming a method for producing an antibody against a fusion polypeptide containing a *Sarcocystis neurona* antigen selected from the group consisting of the 16 (±4) kDa and the 30 (±4) kDa antigen fused to a polypeptide which enables the resulting fusion polypeptide to be isolated by affinity chromatography. This amendment is supported in the specification by the sentence bridging pages 15-16 and the discussion that follows on pages 16-21.

Claims 29 and 30 have also been amended to clarify that the fusion polypeptide comprises a *Sarcocystis neurona* antigen fused to the polypeptide. The polypeptide is of a type which enables the fusion polypeptide to be isolated by affinity chromatography (*See* pages 18-21).

Claims 32, 33, 34, and 35 have been amended to address the concerns of the Examiner. In particular, Claims 32, 33, 34, and 35 have been amended to recite that the polypeptide comprising the fusion polypeptide is protein A (Claim 32), polyhistidine (Claim 33),

-13-

glutathione (Claim 34), or maltose binding protein (Claim 35).

Claims 29 and 30 were rejected because the Examiner indicated that it was unclear whether the term "(±4)" is intended to be a limitation of the claims. The term "(±4)" in Claims 29 and 30 was intended to be a limitation to the molecular weight of the antigens and is not believed to have rendered the claims indefinite. However, the Claims have been amended to remove the parentheses as suggested by the Examiner.

The above amendments to the claims are believed to satisfy the requirements of 35 U.S.C. § 112, second paragraph. Reconsideration of the rejection is requested.

4. Claims 29-35 were rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps.

Claims 29 and 30 have been amended to recite method steps for the production of polyclonal antibodies (Claim 29) or monoclonal antibodies (Claim 30). The amendments are supported by the specification at page 26, beginning at line 27, and Example 1 and are believed to satisfy the requirements of 35 U.S.C. § 112, second paragraph. Reconsideration of the rejection is requested.

-14-

5. Claims 29-35 were rejected under 35 U.S.C. 103(a) as being unpatentable over Liang et al. (Anal. Biochem. 250: 61-65 (1997)) or Liang et al. (Infect. Immun. 66: 1834-1838 (1998)) or Granstrom et al. (J. Vet. Diagn. 5: 88-90 (1993)) in view of Harlow and Lane (Eds. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988)).

The prior art is not believed to establish that the applicants' claimed method is *prima facie* obvious. To establish a *prima facie* case of obviousness over the prior art, all three of the following must be shown: (1) there must be some suggestion or motivation either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings, (2) there must be a reasonable expectation of success, and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations (M.P.E.P. § 2143).

Liang (1997) teaches a method for purification of proteins from *Sarcocystis neurona* by preparative gel electrophoresis of a lysate made from *Sarcocystis neurona*. Liang (1997) showed that the method could be used to purify a 19 kDa antigen and a 30 kDa antigen from *Sarcocystis neurona* lysates. The 19 kDa antigen

-15-

may be the 16 (±4) antigen identified by the applicants. While Liang (1997) teaches using the isolated proteins for microsequencing, Liang (1997) does not suggest that antigens purified by its method are useful for making fusion polypeptides which are then use to make polyclonal or monoclonal antibodies.

Granstrom teaches a 13 kDa antigen (one of eight other *Sarcocystis neurona*-specific antigens) from solubilized *Sarcocystis neurona* merozoites and teaches that the antigen is a surface antigen but does not teach an antigen that is about 30 kDa. The 13 kDa may be the 16 (±4) antigen identified by the applicants. Granstrom suggests that the 13 kDa antigen is a potential candidate for development of a *Sarcocystis neurona*-specific immunoassay. Such an assay would use one or more of the *Sarcocystis neurona*-specific antigens to detect *Sarcocystis neurona*-specific antibodies in horse serum. Granstrom does not suggest that it would be useful to make polyclonal or monoclonal antibodies against a fusion polypeptide comprising any of the eight antigens.

Liang (1998) teaches that antibodies in sera from horses with EPM recognize a 16 kDa antigen and a 30 kDa antigen. Liang (1998) teaches that the 16 kDa antigen is a "strong immunogen in the horse" which "warrant[s] further investigation as [a] candidate

antigen[] for inclusion in vaccines against *S. neurona infection.*" (page 1937, last para.). However, <u>Liang</u> (1998) implies that the 30 kDa antigen would not be a useful candidate for making an antibody because of the following. <u>Liang</u> (1998) teaches that antibodies against the 16 kDa antigen are neutralizing whereas antibodies against the 30 kDa antigen are not neutralizing (page 1836, left col., lines 1-5). <u>Liang</u> (1998) further teaches that "[a]ntibodies to Sn30 are not recognized as specific since a 30-kDa antigen immunoreactive with sera from horses with EPM is found in other *Sarcocystis* spp." (page 1837, first para., last sentence). The above statements about the 30 kDa antigen would suggest to a person skilled in the art that there is little reason to make a fusion polypeptide comprising the 30 kDa antigen which is then used to make polyclonal or monoclonal antibodies against the 30 kDa antigen.

<u>Harlow and Lane</u> merely provides general methods for making polyclonal and monoclonal antibodies and briefly mentions that fusion proteins have been used to make such antibodies. <u>Harlow and Lane</u> provides no information specifically related to making antibodies against any *Sarcocystis neurona* antigens. Therefore, any motivation to one of ordinary skill in the art to make antibodies against particular antigens of *Sarcocystis neurona* must come from <u>Liang</u> (1997), <u>Liang</u>

-17-

(1998), or <u>Granstrom</u>. However, none of the prior art suggests that it would be desirable to make an antibody against any antigen of *Sarcocystis neurona* much less the 16 (±4) kDa antigen or the 30 (±4) kDa antigen as a component of a fusion polypeptide.

In light of the above, it would not have been *prima facie* obvious to make a polyclonal or monoclonal antibody against the 30 (±4) kDa antigen presented in a fusion polypeptide as set forth in the claims. Since the prior art does not teach or suggest that it would be desirable to make a polyclonal or monoclonal antibody against a 30 kDa antigen, there would not have been any motivation for one of ordinary skill in the art to make a polyclonal or monoclonal antibody against the 30 kDa antigen. In particular, when the prior art teaches that antibodies against the 30 kDa antigen are non-specific and non-neutralizing and, therefore, of limited significance (*See* <u>Liang</u> (1998)).

All the prior art has done is merely identify a 30 kDa antigen. It is the applicants who in commonly owned U.S. Patent 6,153,394 to Mansfield *et al.* show that horse antisera contains antibodies which are specific against a 30 (±4) kDa antigen (specification: page 13, lines 16-21; page 29, lines 28-35). Therefore, the claims drawn to a polyclonal or monoclonal antibody against the 30 (±4) kDa antigen presented in a fusion

-18-

polypeptide would not have been *prima facie* obvious to one of ordinary skill in the art.

The prior art is also not believed to have rendered *prima facie* obvious the applicants' claimed method for making a polyclonal or monoclonal antibody against the 16 (±4) kDa antigen as a component of a fusion polypeptide. The prior art does not teach or suggest it would have been desirable to make polyclonal or monoclonal antibodies against the 16 kDa antigen. The prior art goes no further than to merely suggest that because the 16 kDa antigen is a strong immunogen in the horse, it may be a candidate for inclusion in a vaccine against *Sarcocystis neurona* (*See* Liang 1998)). That alone is not sufficient to motivate one of ordinary skill in the art to make a polyclonal or monoclonal antibody against the 16 (±4) kDa antigen, particularly a 16 (±4) kDa antigen which is a component of a fusion polypeptide. Therefore, making a polyclonal or monoclonal antibody against the 16 (±4) kDa antigen, particularly when it is a component of a fusion polypeptide, would not have been *prima facie* obvious in view of the prior art.

In light of the above, Claims 29 and 30 are not believed to be *prima facie* obvious in view of the prior art. Reconsideration of the rejection is requested.

-19-

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attachment is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**."

In view of the above, it is believed that Claims 29-30 and 32-35 are in proper form for allowance. Notice of allowance is requested.

Respectfully,

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<u>**VERSION WITH MARKINGS TO SHOW CHANGES MADE**</u>

<u>In the specification</u>:

The paragraph beginning at page 1, line 5, and ending at page 1, line 8, was amended as follows.

[

<u>STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT</u>

None.]

The paragraph beginning at page 11, line 1, was amended as follows.

These and other objects of the present invention will become increasingly apparent by reference to the following embodiments [and drawings].

Paragraph beginning at page 13, line 1, was amended as follows.

The present invention provides a vaccine that protects equids against *Sarcocystis neurona*. In a preferred embodiment, the vaccine consists of a 16 (±4) kDa antigen and/or 30 (±4) kDa antigen in a subunit

-1-

vaccine. Preferably, the 16 (±4) kDa antigen and/or 30 (±4) kDa antigen are produced in a recombinant bacterium or eukaryote expression vector which produces the proteins which are then isolated to make the vaccine. In another embodiment of the vaccine, the vaccine is a DNA vaccine that comprises a recombinant DNA molecule, preferably in a plasmid, that comprises DNA encoding all or part of the 16 (±4) kDa antigen and/or 30 (±4) kDa antigen. In another embodiment of the vaccine, the recombinant DNA is inserted into a virus vector to provide a live vaccine which is a recombinant DNA virus. In U.S. Serial No. 09/156,954, filed on September 18, 1998, now U.S. Patent 6,153,394 to Mansfield *et al.*, which is hereby incorporated herein by reference, it was disclosed that *Sarcocystis neurona* possesses two unique antigens, a 16 (±4) antigen and a 30 (±4) kDa antigen. These antigens do not react with antibodies from other *Sarcocystis* spp. Thus, these antigens are useful for producing vaccines that protect equids against *Sarcocystis neurona*.

The paragraph beginning at page 29, line 13, was amended as follows.

Therefore, in a Western blot embodiment consisting of *Sarcocystis neurona* antigens resolved by

-2-

gel electrophoresis, a biological sample from a vaccinated equid would contain antibodies that bind only with the 16 (±4) antigen and 30 (±4) kDa antigen whereas a sample from an equid infected with, or exposed to, *Sarcocystis neurona* would contain antibodies that bind with additional *Sarcocystis neurona* antigens. The equine antibodies that are bound are identified by treating the blot with labeled antibodies against equine antibodies. Preferably, the label is selected from the group consisting of alkaline phosphatase, horseradish peroxidase, fluorescent compounds, luminescent compounds, colloidal gold, and magnetic particles. Methods for preparing and analyzing Western blots are well known in the art. In a preferred embodiment, the Western blot is pretreated with non-equine antibodies against a *Sarcocystis* sp. other than *Sarcocystis neurona* wherein the pretreatment prevents binding of equine antibodies to those antigens common to all *Sarcocystis* spp. which can be present in the sample. This method is disclosed in Provisional Patent Application Serial No. 60/120,831, filed on February 19, 1999, now U.S. Serial No. 09/506,630, filed February 18, 2000, which is hereby incorporated herein by reference.

In the Claims:

        Claim 31 was cancelled and Claims 29, 30, 32,

33, 34, and 35 were amended as follows.

-29-(Amended)

A method for producing an antibody against a *Sarcocystis neurona* antigen selected from the group consisting of a 16 ±4 kDa antigen and a 30 ±4 kDa antigen comprising:

5   (a) providing a microorganism [in a culture] containing a DNA encoding a fusion polypeptide [comprising at least one epitope of] in which a *Sarcocystis neurona* antigen selected from the group consisting of [a] the 16 [(±4)] ±4 kDa antigen [and/or]

10   and the 30 [(±4)] ±4 kDa antigen [of *Sarcocystis neurona* and] is fused to a polypeptide [that facilitates] which enables isolation of the fusion polypeptide by affinity chromatography;

        (b) culturing the microorganism in a culture

15   to produce the fusion polypeptide from the DNA;

        (c) isolating the fusion polypeptide from the culture by affinity chromatography;

        (d) admixing the fusion polypeptide isolated by the affinity chromatography with an adjuvant to

20   produce an admixture;

        (e) immunizing a mammal with the admixture containing the fusion polypeptide and the adjuvant which causes the mammal to produce antibodies against the

-4-

fusion polypeptide; and

25 (f) removing serum from the immunized mammal and isolating the antibodies from the serum to produce the antibody against the *Sarcocystis neurona* antigen selected from the group consisting of the 16 ±4 kDa antigen and the 30 ±4 kDa antigen

30 [(d) producing the antibody from the polypeptide].

-30-(Amended)

A method for producing a monoclonal antibody against a *Sarcocystis neurona* antigen selected from the group consisting of a 16 ±4 kDa antigen and a 30 ±4 kDa antigen comprising:

5 (a) providing a microorganism [in a culture] containing a DNA encoding a fusion polypeptide [comprising at least one epitope of] in which a *Sarcocystis neurona* antigen selected from the group consisting of [a] the 16 [(±4)] ±4 kDa antigen [and/or]

10 and the 30 [(±4)] ±4 kDa antigen [of *Sarcocystis neurona* and] is fused to a polypeptide [that facilitates] which enables isolation of the fusion polypeptide by affinity chromatography;

(b) culturing the microorganism in a culture

15 to produce the fusion polypeptide from the DNA;

(c) isolating the fusion polypeptide from the

-5-

culture by the affinity chromatography;

  (d) admixing the fusion polypeptide isolated by the affinity chromatography with an adjuvant to produce an admixture;

  (e) inoculating mice with the admixture containing the fusion polypeptide and the adjuvant which causes the mice to produce antibodies against the fusion polypeptide;

  (f) removing the spleens from the mice which produce the antibodies against the fusion polypeptide;

  (g) removing spleen cells from the spleens and mixing the spleen cells from the spleens with mouse myeloma cells to produce a mixture of fused cells consisting of spleen cells fused to myeloma cells, the spleen cells, and the myeloma cells;

  (h) selecting the fused cells on cell culture medium in which the fused cells can grow but in which the spleen cells and the myeloma cells cannot grow; and

  (i) screening the fused cells for fused cells which produce the monoclonal antibody against the *Sarcocystis neurona* antigen selected from the group consisting of the 16 ±4 kDa antigen and the 30 ±4 kDa antigen to produce the monoclonal antibody

  [(d) producing the monoclonal antibody from the polypeptide].

-32-(Amended)

The method of Claim 31 wherein the polypeptide comprising the fusion polypeptide is [all or a portion of] protein A and the affinity chromatography comprises an IgG-linked resin.

-33-(Amended)

The method of Claim 31 wherein the polypeptide comprising the fusion polypeptide is polyhistidine and the affinity chromatography comprises a Ni^{2+} resin.

-34-(Amended)

The method of Claim 31 wherein the polypeptide comprising the fusion polypeptide is glutathione S-transferase and the affinity chromatography comprises a glutathione Sepharose 4B resin.

-35-(Amended)

The method of Claim 31 wherein the polypeptide comprising the fusion polypeptide is a maltose binding protein and the affinity chromatography comprises an amylose resin.